SUPPLEMENTARY MATERIAL

A New *Ent*-Kaurane Diterpenoid from *Ixora amplexicaulis*

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A New *Ent*-Kaurane Diterpenoid from *Ixora amplexicaulis*

A new *ent*-kaurane diterpenoid, $6\alpha,16\alpha$ -dihydroxy-*ent*-kaurane (**1**), was isolated from the stems of *Ixora amplexicaulis*, together with (24*R*)- 6β -hydroxy-24-ethyl-cholest-4-en-3-one (**2**), 7β -hydroxysitosterol (**3**), maslinic acid (**4**), 3,3'-bis(3,4-dihydro-4-hydroxy-6-methoxy-2*H*-1-benzopyran) (**5**) and protocatechuric acid (**6**). Their structures were mainly established by extensive spectroscopic analysis, including 2D-NMR techniques. **2-5** were isolated from the genus *Ixora* for the first time and **6** obtained originally from *I. amplexicaulis*.

Keywords: *Ixora amplexicaulis*; Rubiaceae; *ent*-kaurane diterpenoid

Supporting information

Spectroscopic data of the known compounds **2-6**.

Figure S1. Key HMBC, COSY and ROESY correlations of **1**.

Original spectra of compound **1**.

^1H NMR Spectrum (400 MHz, CDCl_3)

^{13}C NMR Spectrum (100 MHz, CDCl_3)

COSY Spectrum

HSQC Spectrum

HMBC Spectrum

HREIMS

EIMS

IR

Spectroscopic data of the known compounds **2-6**.

(24R)-6 β -Hydroxy-24-ethyl-cholest-4-en-3-one (2)

White powder, C₂₉H₄₈O₂, MW: 428; EI-MS m/z: 428 (M⁺); ¹H NMR (CDCl₃, 500 MHz): δ 5.81 (1H, s, H-4), 4.34 (1H, d, J = 2.7, H-6), 1.68-1.22 (7H, dd, -CH-H), 1.05-1.17 (20H, -CH₂-H'), 0.96 (3H, d, J = 6.4, H-21), 0.85 (3H, dd, J = 7.0, 7.0, H-24''), 0.83 (3H, d, J = 7.0, H-27), 0.81 (3H, d, J = 7.0, H-26), 0.76 (3H, s, H-19), 0.74 (3H, s, H-18). ¹³C NMR (CDCl₃, 500 MHz): δ 200.8 (C-3), 168.5 (C-5), 126.2 (C-4), 73.2 (C-6), 56.0 (C-14), 55.8 (C-17), 53.6 (C-9), 45.8 (C-24), 42.5 (C-13), 39.5 (C-12), 38.5 (C-7), 38.0 (C-10), 37.0 (C-1), 36.0 (C-20), 34.2 (C-2), 33.9 (C-22), 29.6 (C-8), 29.1 (C-25), 28.1 (C-16), 26.0 (C-23), 24.1 (C-15), 23.0 (C-24'), 20.9 (C-11), 19.7 (C-26), 19.4 (C-19), 19.2 (C-27), 19.0 (C-13), 18.7 (C-21), 11.9 (C-18), 11.8 (C-24'').

7 β -Hydroxysitosterol (3)

White powder, C₂₉H₅₀O₂, MW: 430; EI-MS m/z: 430 (M⁺); ¹H NMR (CDCl₃, 400 MHz): δ 5.30 (1H, s, H-6), 3.56 (1H, m, H-3 α), 3.86 (1H, d, J = 4.5, H-7 α), 1.05 (3H, s, H-19), 0.93 (3H, d, J = 6.6, H-21), 0.85 (3H, d, J = 6.6, H-26), 0.81 (3H, d, J = 6.6, H-27), 0.85 (3H, t, J = 7.2, H-29), 0.70 (3H, s, H₃-18); ¹³C NMR (CDCl₃, 400 MHz): δ 143.4 (C-5), 125.4 (C-6), 73.3 (C-7), 71.4 (C-3), 55.9 (C-17), 55.3 (C-14), 48.2 (C-9), 45.8 (C-24), 41.7 (C-4), 40.9 (C-8), 39.5 (C-12), 36.8 (C-1), 36.1 (C-10), 33.9 (C-22), 31.5 (C-2), 29.1 (C-25), 28.5 (C-16), 26.3 (C-15), 26.3 (C-15), 26.0 (C-23), 23.0 (C-28), 21.0 (C-11), 19.8 (C-26), 19.1 (C-19), 18.9 (C-21), 11.9 (C-29), 11.8 (C-18).

Maslinic acid (4)

White powder, C₃₀H₄₈O₄, MW: 472, EI-MS m/z: 472 (M⁺); ¹H NMR (CDCl₃, 500 MHz): δ 5.24 (1H, br s, H-12), 3.60 (1H, m, H-2), 2.89 (1H, d, J = 9.6, H-3), 2.84 (1H,

m, H-18), 1.15 (3H, s), 1.02 (3H, s), 1.01 (6H, s), 0.99 (3H, s), 0.92 (3H, s), 0.84 (3H, s), 0.80 (3H, s); ^{13}C NMR (CDCl_3 , 500 MHz): δ 181.8 (C-28), 145.4 (C-13), 123.5 (C-12), 84.5 (C-3), 69.5 (C-2), 56.7 (C-5), 48.5 (C-1), 48.4 (C-9), 47.7 (C-17), 47.3 (C-19), 43.5 (C-14), 43.0 (C-18), 40.7 (C-4), 40.6 (C-8), 39.3 (C-10), 35.0 (C-21), 34.0 (C-29), 33.9 (C-22), 33.7 (C-7), 31.6 (C-20), 29.3 (C-23), 28.8 (C-15), 26.4 (C-27), 24.6 (C-16), 24.0 (C-11), 24.0 (C-30), 19.6 (C-6), 17.8 (C-24), 17.5 (C-26), 17.1 (C-25).

3,3'-Bis(3,4-dihydro-4-hydroxy-6-methoxy-2H-1-benzopyran) (5)

Yellow-green oil, $\text{C}_{20}\text{H}_{22}\text{O}_6$, MW: 358; ^1H NMR (CD_3Cl , 500 MHz): δ 6.89 (2H, d, $J=8.1$, H-8,8'), 6.88 (2H, d, $J=1.8$, H-5,5'), 6.82 (2H, dd, $J=8.4, 1.8$, H-7,7'), 4.74 (2H, d, $J=4.3$, H-4,4'), 4.24 (2H, dd, $J=9.1, 6.8$, H-2), 3.88 (2H, dd, $J=9.1, 3.8$, H-2'), 3.88 (6H, s, C-6,6'-OMe), 3.11 (2H, m, $J=8.1, 3.8$, H-3,3'); ^{13}C NMR (CD_3Cl , 500 MHz): δ 146.7 (C-6,6'), 145.2 (C-9,9'), 132.9 (C-10,10'), 118.9 (C-7,7'), 114.3 (C-5,5'), 108.6 (C-8,8'), 85.8 (C-4,4'), 71.6 (C-2,2'), 55.9 (C-6,6'-OMe), 54.1 (C-3,3').

Protocatechuric acid (6)

White powder, $\text{C}_7\text{H}_6\text{O}_4$, MW: 154; ^1H NMR [$(\text{CD}_3)_2\text{O}$, 500 MHz]: δ 7.51 (1H, d, $J=2.4$, H-2), 7.46 (1H, dd, $J=8.2, 1.8$, H-6), 6.88 (1H, d, $J=8.0$, H-5); ^{13}C NMR [$(\text{CD}_3)_2\text{CO}$, 400 MHz]: δ 167.6 (-COOH), 150.6 (C-4), 145.4 (C-3), 123.5 (C-6), 123.0 (C-1), 117.3 (C-2), 115.6 (C-5).

Figure S1. Key HMBC, COSY and ROESY correlations of **1**.

Original spectra of compound **1**.

^1H NMR:

¹³C NMR:

Original spectra of compound **1**.

^1H - ^1H COSY:

Original spectra of compound **1**.

HSQC:

Original spectra of compound **1**.

HMBC:

Original spectra of compound **1**.

ROESY:

Original spectra of compound 1.

HREIMS:

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -10.0, max = 120.0

Selected filters: None

Monoisotopic Mass, Odd and Even Electron Ions

16 formula(e) evaluated with 1 results within limits (up to 51 closest results for each mass)

Elements Used:

C: 0-200 H: 0-400 O: 1-3

Nci-14

15:18:58 08-Nov-2013

Voltage EI+

KIE
M131108EA-05AFANM 12 (1.102)
306.2558

Autospec Premier
P776
115

Minimum: -10.0
Maximum: 120.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
306.2558	306.2559	-0.1	-0.3	4.0	5546070.5	C20 H34 O2

Original spectra of compound **1**.

EIMS:

Original spectra of compound **1**.

IR: